



RESEARCH PAPER

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A comparative study of bioactive potentials in pulp and peel of unripe *Carica papaya* L. fruit

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Abstract

This study investigates the comparative antibacterial and antioxidant potentials of the pulp and peel of unripe *Carica papaya* L. fruit. Methanol and acetone extracts of both parts were evaluated for their activity against multiple drug-resistant bacteria (*Escherichia coli*, *Klebsiella* sp, *Salmonella* sp., and *Shigella* sp.) using the disc diffusion method. The peel exhibited a higher antibacterial activity with a maximum zone of inhibition of 16.2 mm compared to the pulp's 14.4 mm; however, standard Kanamycin (30 µg/disc) showed >30 mm. Minimum inhibitory concentration (MIC) values ranged from 200-300 mg/ml for peel and 250-350 mg/ml for pulp, while minimum bactericidal concentration (MBC) was also lower in peel (500-600 mg/ml) than pulp (550-700 mg/ml). Furthermore, the peel demonstrated superior antioxidant activity with a DPPH scavenging rate of 23.02%, compared to the pulp's 5.34%, although ascorbic acid exhibited 100% scavenging at 100 µg/ml. Peel also showed a lower IC₅₀ value (61.035 µg/ml) than pulp (150.466 µg/ml) in DPPH assay, while ascorbic acid had an IC₅₀ of 37.062 µg/ml. Total phenolic content was higher in peel (32.50 mgL⁻¹ GAE/g dry material) than pulp (21.25 mgL⁻¹ GAE/g dry material). These findings suggest that papaya peel is richer in bioactive compounds and could be effectively utilized for its antibacterial and antioxidant properties, highlighting its potential as a valuable natural resource rather than being wasted.

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Introduction

The emergence of multidrug-resistant (MDR) pathogens and the increasing prevalence of oxidative stress-related diseases have intensified global efforts to identify plant-derived bioactive compounds as safer and more sustainable alternatives to synthetic pharmaceuticals (Ventola, 2015). Fruits especially those consumed in both ripe and unripe forms are not only appreciated for their nutritional value but also increasingly recognized for their therapeutic potential, including antimicrobial and antioxidant properties (Sagar *et al.*, 2018).

Carica papaya L. (papaya) is a widely cultivated tropical fruit renowned for its medicinal and nutritional benefits. Traditionally, various parts of the papaya plant such as leaves, seeds, roots, latex, and fruits have been employed in folk medicine to treat ailments including gastrointestinal disorders, infections, inflammation, and skin conditions (Krishna *et al.*, 2008). The unripe fruit, in particular, is rich in bioactive constituents such as alkaloids, flavonoids, tannins, and phenolic compounds, which are known to possess potent antibacterial and antioxidant effects (Maisarah *et al.*, 2014).

While the edible pulp of papaya is commonly consumed, its non-edible parts, particularly the peel, are frequently discarded as agricultural waste. However, emerging research suggests that fruit peels may be rich in phytochemicals, presenting an untapped source of bioactive agents with significant health benefits (Maisarah *et al.*, 2014). Plant-derived antioxidants can neutralize free radicals and thereby reduce the risk of chronic diseases such as cancer, cardiovascular disorders, and neurodegenerative conditions. Similarly, natural antibacterial agents from plants have shown promise in addressing the growing challenge of antibiotic-resistant infections (Insanu *et al.*, 2022).

Although considerable research has focused on *C. papaya*, few studies have compared the bioactive properties of its edible and non-edible components, particularly in the unripe stage, which may contain

higher levels of certain phytochemicals. Therefore, this study aims to evaluate and compare the antibacterial and antioxidant activities of the edible (pulp) and non-edible (peel) parts of unripe *Carica papaya* fruit. Through standardized extraction methods and established bioassays, this investigation seeks to highlight the bioefficacy of both components and assess the potential of papaya peel as a valuable and sustainable resource for pharmaceutical or nutraceutical applications.

Materials and methods

Plant material

Unripe papaya (*Carica papaya*) fruits were collected from Saheb Bazar, a local market in Rajshahi, Bangladesh. The fruits were confirmed to be free from chemical treatment at the time of collection. The sample was identified and authenticated as *Carica papaya* (L.) by Dr. AHM Mahbubur Rahman, Professor and Plant Taxonomist, Department of Botany, University of Rajshahi. A voucher specimen was deposited at the department's Herbarium for future reference.

The freshly collected papaya fruits were washed thoroughly with distilled water to remove surface impurities. Using a sterilized knife, the fruits were carefully separated into edible (whole fruit – peel = pulp) and non-edible (whole fruit – pulp = peel) parts. Each part was chopped into small pieces and air-dried at room temperature (32–35°C) for five days until a constant weight was achieved. A total of 250g from each part was coarsely ground using a mortar and pestle, followed by fine grinding using an electric blender. The resulting powdered materials (edible and non-edible) were stored separately in airtight bottles for future use.

Extraction procedures

Carica papaya fruit extracts were prepared following the method of Sultana *et al.* (2009), with slight modifications. Each portion of the powdered, air-dried plant material (edible and non-edible parts) was extracted separately using methanol and acetone as solvents. For each extraction, 5g of powdered sample

was mixed with 100 ml of the respective solvent in a conical flask and allowed to soak at ambient temperature for 24 hours.

The soaked mixtures were first filtered through tetron cloth into beakers, followed by filtration using Whatman No. 1 filter paper. The resulting filtrates were concentrated at 40°C using a rotary evaporator (EYELA, SB-651, Rikakikai Co. Ltd., Tokyo, Japan). The concentrated extracts were then transferred to screw-cap tubes and stored at 4°C for future use. The yield was found to be 5.67±2.58 and 6.94±1.76 w/w in methanol and acetone, with reference to the air dried powdery plant material. The dried extracts were dissolved in dimethylsulphoxide and subjected to antibacterial activity.

Test organisms

Four species of multiple drug-resistant (MDR) bacteria - *Escherichia coli*, *Klebsiella* sp., *Salmonella* sp., and *Shigella* sp. were used in this study. Clinical isolates were obtained from the Plant Biotechnology and Microbiology Laboratory of the Department of Botany, Rajshahi University. The organisms were periodically sub-cultured and maintained on nutrient agar slants at 4°C.

Antibacterial activities

The antibacterial activity of the methanolic and acetonic extracts of the plant sample was evaluated using the disc diffusion method according to Bauer *et al.* (1966) with slight modifications. Bacterial cultures were adjusted to 0.5 McFarland turbidity standards and inoculated onto Nutrient agar (Oxoid) plates (diameter: 15cm). Sterile filter paper discs (diameter 6mm) impregnated with 100µl of extract dilutions reconstituted in the minimum amount of solvent at concentrations of 50 and 100mg/ml were applied to each of the culture plates previously seeded with the bacterial cultures. After incubation at 37°C for 24 hours, the diameters of the clear zones on the agar surface were measured to the nearest millimeter using a ruler. Values <8 mm were considered inactive against microorganisms (Bhalodia and Shukla, 2011). The experiment was replicated three times for

reproducibility. Commercially available kanamycin discs (30 µg/disc) were used as positive controls, while discs prepared with the appropriate solvents served as negative controls.

Determination of Minimum Inhibitory Concentration and Minimum Bactericidal Concentration

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined according to Doughari *et al.* (2007) with slight modifications. The MIC of each extract was determined against each test bacterium in concentrations ranging from 100 to 700 mg/ml. Tubes containing nutrient broth (9ml) were inoculated with 1ml of each test organism previously diluted to 0.5 McFarland turbidity standards and incubated at 37°C for 24 hours. MIC was determined as the concentration at which no turbidity change was observed.

Determination of Antioxidant Activity using Free Radical Scavenging Activity (DPPH)

The free radical scavenging activity of the extracts against 1,1-diphenyl-2-picrylhydrazyl (DPPH) was determined following the method of Braca *et al.* (2001). Plant extract (200 µl) at different concentrations (5, 25, 50, 100, 500 µg/ml) was mixed with 2 ml of a 0.004% methanol solution of DPPH. After 30 minutes, absorbance was measured at 517 nm using a UV spectrophotometer (Shimadzu, UV-1601PC) against a blank. The percentage scavenging activity of the extracts was calculated using the formula: % scavenging activity = $\{(A_0 - A_1) / A_0\} \times 100$; where A_0 is the absorbance of the control and A_1 is the absorbance of the extract or standard.

Determination of IC₅₀ Value

A percent inhibition versus concentration curve was plotted and the concentration of sample required for 50 percent inhibition was determined and expressed as IC₅₀ value. The lower the IC₅₀ value indicates high antioxidant capacity. The effective concentration of sample required to scavenge DPPH radical by 50% (IC₅₀ value) was obtained by linear regression

analysis of dose-response curve plotting between % inhibition and concentrations.

Determination of total phenolic content

Total phenolic content was estimated using the Folin-Ciocalteu (F-C) reagent spectrophotometric method described by Ainsworth and Gillespie (2007) with slight modifications. Gallic acid was used as a standard material for creating the calibration curve. The assay relies on electron transfer reactions from phenolic compounds to phosphomolybdic or phosphotungstic acid complexes, measured spectroscopically at 765 nm.

Results and discussion

In vitro antibacterial activity

The antibacterial potential of methanolic and acetonitrile extracts from the edible (pulp) and non-edible (peel) parts of unripe *Carica papaya* fruit was assessed *in vitro* using the disc diffusion method against four multidrug-resistant (MDR) bacterial strains: *Escherichia coli*, *Klebsiella* sp., *Salmonella* sp., and *Shigella* sp. (Figure 1.). The inhibition zones produced by the extracts were compared with the standard broad-spectrum antibiotic Kanamycin (30 µg/disc). Table 1 summarizes the mean inhibition zones observed.

Table 1. Antibacterial activities of Edible and Non-edible Parts of *M. sapientum* fruit extracts against selected MDR bacteria.

Used Sample (Part of banana)	Concentration (mg/ml)	Zone of inhibition (mm)							
		Methanol				Acetone			
		<i>Escherichia coli</i>	<i>Klebsiella</i>	<i>Salmonella</i>	<i>Shigella</i>	<i>Escherichia coli</i>	<i>Klebsiella</i>	<i>Salmonella</i>	<i>Shigella</i>
Edible Part	200	+	+	+	+	+	+	+	+
Non- Edible Part		+	+	+	+	+	+	+	+
Edible Part	400	10.7±0.21	9.5±0.73	9.6±0.52	8.7±0.67	8.8±0.24	8.1±0.21	8.2±0.82	+
Non- Edible Part		11.6±0.47	10.4±0.58	10.9±0.39	9.2±0.66	9.2±0.78	9.1±0.48	8.8±0.24	8.2±0.88
Edible Part	600	12.7±0.63	11.7±0.78	12.7±0.36	10.1±0.48	10.8±0.21	10.3±0.34	10.7±0.45	9.9±0.65
Non- Edible Part		12.9±0.67	11.9±0.57	13.2±0.88	12.1±0.37	11.3±0.60	10.4±0.55	10.5±0.50	10.6±0.44
Edible Part	800	13.9±0.55	13.5±0.94	14.4±0.32	11.9±0.67	12.8±0.39	10.4±0.66	11.2±0.48	10.7±0.27
Non- Edible Part		14.2±0.65	14.5±0.88	16.2±0.41	13.9±0.59	13.3±0.75	12.4±0.95	13.6±1.20	13.5±0.48
Negative Control		+	+	+	+	+	+	+	+
Positive Control (Kanamycine 30µg/disc)		>30	>30	>30	>30	>30	>30	>30	>30

Data are represented as mean ± SD of triplicate experiments. + = Bacterial growth.

ANOVA for the Table 1.

Item	SS	df	MS	F
Sample	14.63	1	14.63	9.97
Solvent	35.40	1	35.40	24.11
concentration	1632.80	3	544.27	370.71
Bacteria	20.97	3	6.99	4.76
Error	80.75	55	1.47	
Total	1784.56	63	28.33	

The papaya fruit extracts produced inhibition zones ranging from 8.1 mm to 16.2 mm, whereas the standard antibiotic Kanamycin exhibited significantly larger zones of inhibition (>30 mm) across all tested bacterial species. Among the extracts, the peel (non-edible part) demonstrated greater antibacterial

activity than the pulp (edible part) in both methanol and acetone extractions. Notably, the methanolic extract of the peel showed the highest inhibition zone of 16.2 mm against *Salmonella* sp. at 800 mg/mL, compared to 14.4 mm by the pulp. This trend was consistent at lower concentrations: at 600 mg/mL

and 400 mg/mL, the peel extracts produced inhibition zones of 13.2 mm and 11.6 mm, respectively, while the pulp showed 12.7 mm and 10.7 mm. At 200 mg/mL, neither the peel nor pulp extracts demonstrated antibacterial activity,

indicating a concentration-dependent response. Negative controls containing only solvents showed no inhibition, confirming the antimicrobial activity was solely due to the phytochemical content of the extracts.

Table 2. MIC and MBC value of edible and non-edible part of *M. sapientum* fruit extracts (methanolic and acetonic) against selected MDR bacteria.

Used Part	MIC value (mgml ⁻¹)							
	<i>Escherichia coli</i>		<i>Klebsiella</i> sp.		<i>Salmonella</i> sp		<i>Shigella</i> sp.	
	Methanol	Acetone	Methanol	Acetone	Methanol	Acetone	Methanol	Acetone
Edible Part	250	300	300	350	250	300	300	350
Non- Edible Part	200	250	250	250	200	250	250	300
Used Part	MBC value (mgml ⁻¹)							
	Methanol	Acetone	Methanol	Acetone	Methanol	Acetone	Methanol	Acetone
Edible Part	550	600	600	650	550	600	650	700
Non- Edible Part	500	550	550	550	500	550	550	600

ANOVA for MIC and MBC for Table 2.

Item	Minimum inhibitory concentration (MIC)				Minimum bactericidal concentration (MBC)			
	SS	df	MS	F	SS	df	MS	F
Used Part	12656.25	1	12656.25	81	18906.25	1	18906.25	67.22
Solvent	7656.25	1	7656.25	49	7656.25	1	7656.25	27.22
Bacteria	7968.75	3	2656.25	17	15468.75	3	5156.25	18.33
Error (within)	1562.5	10	156.25		2812.5	10	281.25	
Total	29843.75	15			44843.75	15		

Statistical analysis using ANOVA confirmed that the peel extracts produced significantly larger zones of inhibition compared to pulp extracts ($p < 0.05$), affirming the superior antibacterial efficacy of the non-edible part. This aligns with previous findings by Akinmoladun *et al.* (2020), who reported higher antibacterial activity in papaya peel than pulp against MDR bacteria. Similarly, Vasundra and Upma (2024) demonstrated notable inhibition zones from ethanolic extracts of papaya peel and seed against *E. coli*, *Klebsiella pneumoniae*, *Bacillus cereus*, and *Staphylococcus aureus*.

In terms of solvents, methanol extracts exhibited consistently stronger antibacterial effects than acetone extracts across both peel and pulp samples. This supports earlier research by Dhawan and Gupta (2017), which emphasized methanol's superior efficiency in extracting active phytochemicals such as

flavonoids, tannins, and phenolics, all known for their antimicrobial properties.

Collectively, these findings suggest that the non-edible papaya peel, especially when extracted with methanol, holds significant promise as a natural antibacterial agent and could be explored further for pharmaceutical applications.

MIC and MBC for Edible and Non-edible part of Carica papaya

The results of screening MIC and MBC values of edible and non-edible part of unripe papaya fruit extracts are presented in Table 2. The MIC values across all samples ranged from 200 to 350 mg/mL, while MBC values ranged from 500 to 700 mg/mL. Specifically, the MIC values for pulp extracts were found to be between 250–350 mg/mL and MBC between 550–700 mg/mL.

Table 3. Determination DPPH radical inhibition or scavenging assay/activity.

<i>Musa sapientum</i>	Abs (sample) [A _i]	Abs [blank]	Abs (control) [A _o]	DPPH scavenging /inhibition activity		
				[I(%) = 100 x (A _o -A _i)/A _o]		
				Mean	±	SD
Edible part	0.4269	0	0.451	5.34 ± 0.11		
Non-edible part	0.3472	0	0.451	23.02 ± 0.78		
<i>Ascorbic acid (standard)</i>	0.0000	0	0.451	100 ± 0.00		

In contrast, the peel extracts demonstrated lower MIC values (200–300 mg/mL) and MBC values (500–600 mg/mL), indicating higher antibacterial efficacy. Between the two solvents tested, methanol consistently yielded lower MIC and MBC values compared to acetone. This suggests that methanol is

more efficient in extracting bioactive compounds responsible for antibacterial activity—an observation supported by previous studies demonstrating methanol's effectiveness in solubilizing polar phytochemicals such as phenolics and flavonoids (Cowan 1999 and Dhawan; and Gupta, 2017).

Table 4. Total Phenols content of the edible and non-edible part of papaya.

<i>Carica papaya</i>	Total Phenols [mgL ⁻¹ GAE / g dry material]
	Mean ± SD
Edible part	21.25 ± 1.78
Non-edible part	32.50 ± 1.25

The variability in MIC and MBC values highlights differences in the susceptibility of bacterial strains to plant-derived antimicrobials. Lower MIC and MBC values correspond to higher antibacterial potency. The non-edible peel part exhibited significantly stronger antibacterial activity than the edible pulp, as evidenced by consistently lower MIC and MBC values. This trend reinforces earlier findings from Akinmoladun *et al.* (2020), which also showed that papaya peel extracts were more effective than pulp against MDR bacterial strains.

These results support the idea that peels, often treated as waste, can be a valuable source of antimicrobial agents. Several studies have reported similar findings in other fruits, where peel fractions demonstrated greater antimicrobial activity than their pulp counterparts, due to higher concentrations of secondary metabolites like tannins, alkaloids, and polyphenols (Sagar *et al.*, 2018 and Akinmoladun *et al.*, 2020). Based on these findings, it can be concluded that methanolic extracts of unripe *Carica papaya* peel possess broad-spectrum antibacterial activity and may be a promising candidate for

developing natural antimicrobial agents, especially in the fight against multidrug-resistant bacteria.

Analysis of Antioxidant Activities and IC₅₀ Value

The antioxidant potential of methanolic and acetic extracts from the edible (pulp) and non-edible (peel) parts of unripe *Carica papaya* was evaluated using the DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging assay, a widely used method for assessing free radical quenching in plant extracts (Insanu *et al.*, 2022; Vasundra and Upma, 2024).

Results indicated a concentration-dependent increase in antioxidant activity (Table 3 and Figure 2). At 100 µg/mL, the peel extract exhibited higher DPPH scavenging activity (23.02%) than the pulp (5.34%), while the standard, ascorbic acid, showed complete inhibition (100%).

The IC₅₀ values further confirmed this trend: the peel extract had a lower IC₅₀ (61.035 µg/mL) than the pulp (150.466 µg/mL), indicating greater antioxidant efficiency. Ascorbic acid had the most potent effect (IC₅₀ = 37.062 µg/mL).

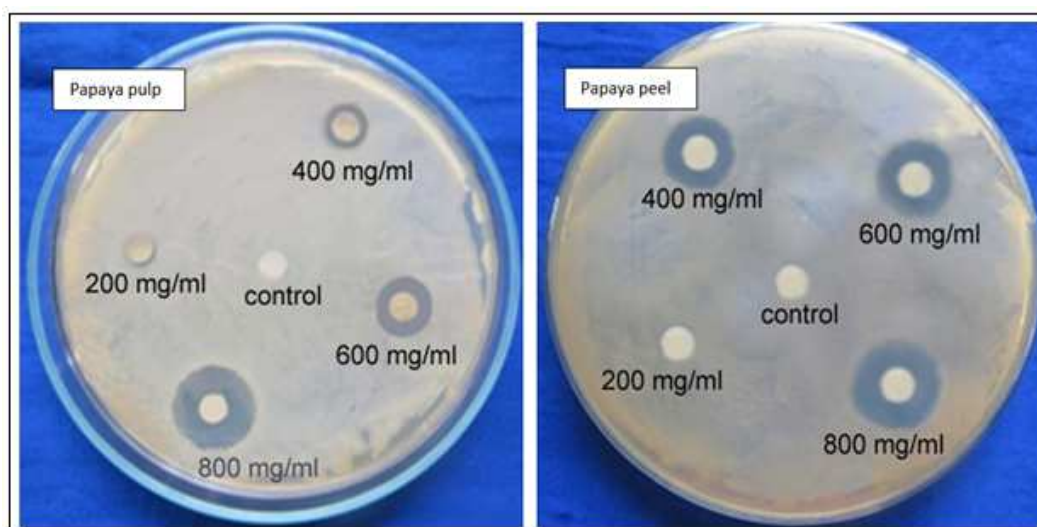


Fig. 1. Antibacterial activity of edible and non-edible part of *C. papaya* extract against *E. coli*.

These findings align with Vasundra and Upma (2024), who reported higher antioxidant performance in papaya peel than seed extracts. Similarly, Insanu *et al.* (2022) found antioxidant activity index (AAI) values ranging from 1.91 to 47.89 in *C. papaya*, with

peel and seed extracts outperforming the pulp. The high antioxidant activity was attributed to the presence of phenolic and flavonoid compounds, particularly in the ethyl acetate and n-hexane extracts of peel and seed, respectively.

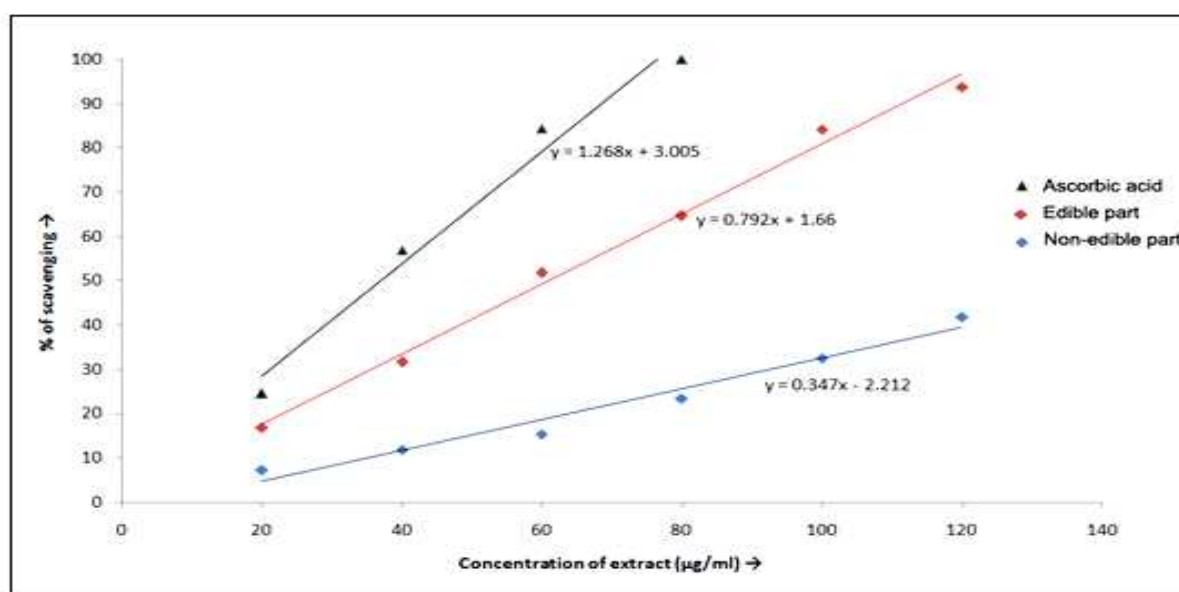


Fig. 2. Calibration curve Determination of IC_{50} value of edible and non-edible part of *C. papaya* extract along with Ascorbic acid.

Estimation of total phenols content

The total phenolic content (TPC), including flavonoids, of the edible (pulp) and non-edible (peel) parts of unripe *Carica papaya* was determined using the Folin-Ciocalteu (F-C) spectrophotometric

method. Gallic acid was used as the calibration standard, and the results are expressed in mg gallic acid equivalents (GAE) per gram of dry material (Table 4).

The analysis revealed that the peel had a significantly higher TPC (32.50 ± 1.25 mg GAE/g) than the pulp (21.25 ± 1.78 mg GAE/g). These findings suggest that the peel, often discarded as waste, contains a richer reserve of polyphenolic compounds than the commonly consumed pulp. This is consistent with prior research of Parvez *et al.* (2020) indicating that non-edible parts of fruits often possess higher concentrations of bioactive compounds. Insanu *et al.* (2022) reported similar results in papaya, where the highest TPC and total flavonoid content (TFC) were found in the n-hexane extract of papaya seeds and ethyl acetate extract of the flesh, respectively. Additionally, Vasundra and Upma (2024) observed a TPC of 62.45 mg GAE/g dry weight in papaya seeds, highlighting the strong antioxidant potential of non-edible parts. Phenolic compounds are well-known for their antioxidant, antimicrobial, and anti-inflammatory properties. Therefore, the higher TPC in papaya peel suggests its potential as a valuable source of natural antioxidants suitable for pharmaceutical and nutraceutical applications.

Conclusion

This study evaluated the *in vitro* antimicrobial and antioxidant properties of the edible (pulp) and non-edible (peel) parts of unripe *Carica papaya* fruit. Both methanolic and acetonetic extracts exhibited notable antibacterial activity against multidrug-resistant strains of *Escherichia coli*, *Klebsiella* spp., *Shigella* spp., and *Salmonella* spp. Additionally, the extracts demonstrated significant antioxidant activity, with higher concentrations of phenolic compounds particularly evident in the peel. Comparative analysis revealed that the non-edible peel consistently outperformed the pulp in both antibacterial and antioxidant assays. These findings highlight the potential of papaya peel as a valuable, underutilized source of bioactive compounds. Utilizing such agricultural by-products not only adds value but also supports sustainable and eco-friendly approaches for developing natural antioxidant and antibacterial formulations in the pharmaceutical and nutraceutical industries.

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